The continuum of consciousness in cardiovascular stress research : an experimental expedition

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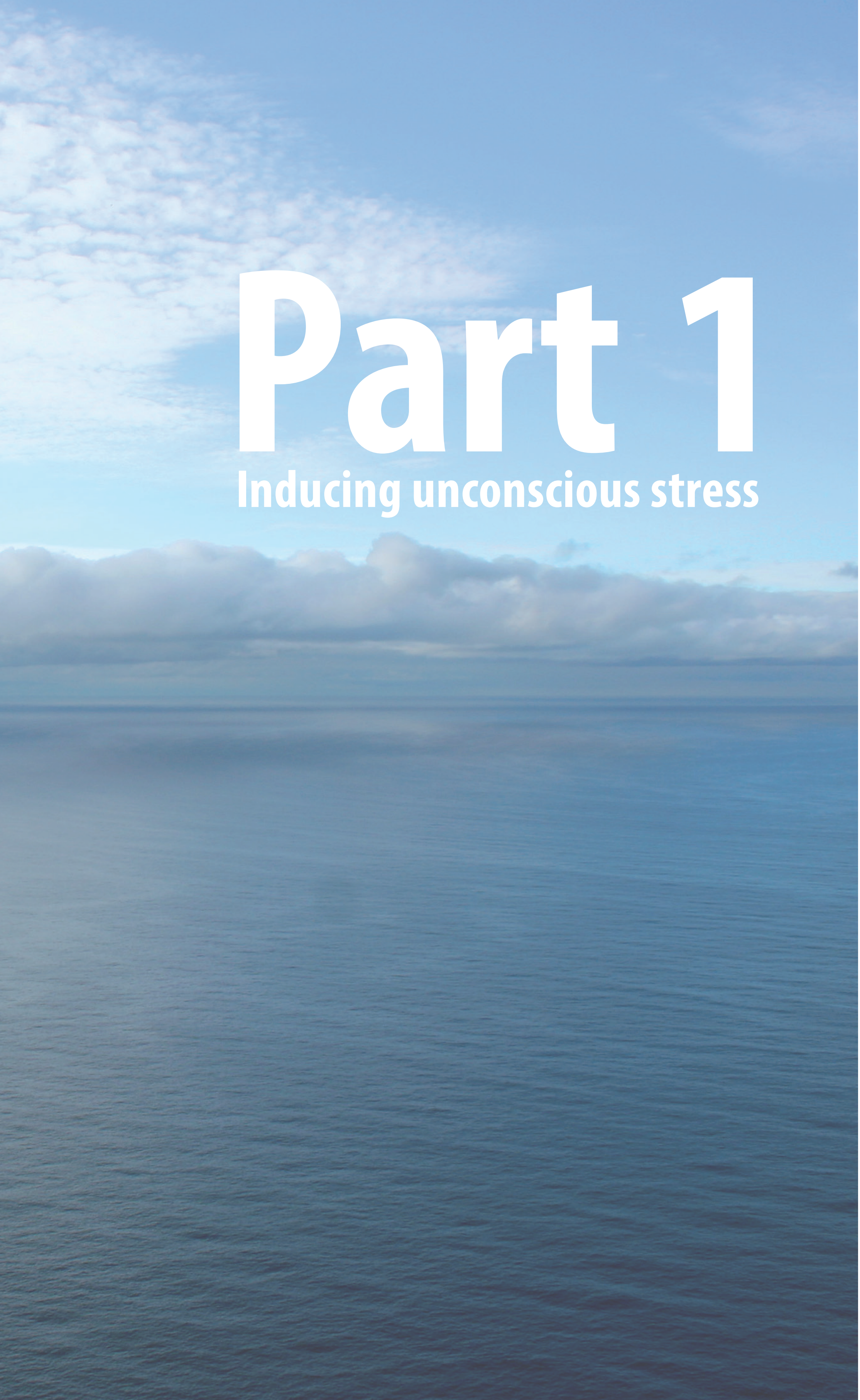
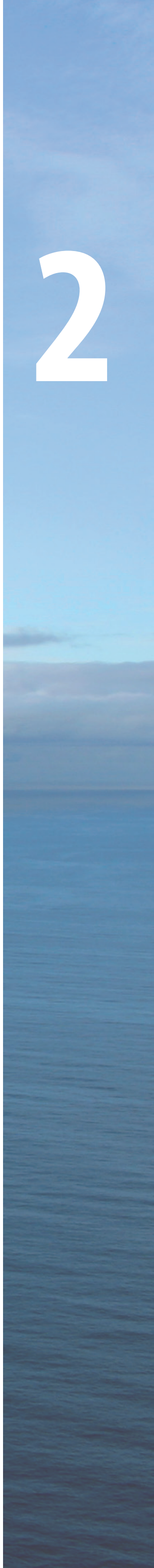


Part 1

Inducing unconscious stress

Chapter 2



Peripheral physiological responses to subliminally presented negative affective stimuli: A systematic review

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Abstract

Negative affective information may be presented outside of awareness and change physiological activity. By increasing peripheral physiological activity, subliminally presented negative affective information may contribute to the development of disease. The current systematic review evaluated 65 studies in which negative affective stimuli were presented subliminally to a healthy sample while cardiovascular, electrodermal, electromyographical, hormonal, or immunological activity was measured. Overall, 41% of the tested contrasts indicated significant increases due to negative affective stimuli compared to control stimuli. These effects were most pronounced in fear conditioning studies measuring skin conductance response amplitude and priming studies measuring systolic blood pressure. However, across the included studies the methodology varied substantially and the number of contrasts per physiological parameter was limited. Thus, although some evidence exists that subliminally presented negative affective stimuli can induce adverse peripheral physiological changes, this has not yet been addressed sufficiently.

Can information that occurs outside of awareness affect perception, motivation, decisions, and emotions? Research addressing this question is flourishing in various fields within psychology, including organizational (e.g., 81), emotion (e.g., 47), clinical (e.g., 59), cognitive (53), and social psychology (e.g., 66,104). Surprisingly, the potential role of unconscious processes in the relationship between negative affective information and health has remained understudied. In psychosomatic research, the limits of conscious awareness have long been of interest and explored (28). For example in the 1930s, a psychoanalytic approach was used to address unconscious emotional conflict in the etiology of hypertension (36), but experimental tests of this particular method failed to provide supportive evidence (28). Notwithstanding, the possible adverse influence of negative affective information outside of awareness on physiological systems is consistent with current theoretical insights (26-28,105,106). However, experimental evidence is still scarce. Given that several studies indeed showed that unconscious processes influence the experience of emotions (e.g., 107,108) and behavior (e.g., 57,109) it seems crucial to examine whether physiological parameters can be affected by negative affective stimuli when these are presented outside of awareness.

In fact, the quest for evidence of this kind appears to have a long history. In the early days of psychological research, Jung (1907, 49) and Peterson and Jung (1907, 50) performed several studies regarding the effect of word-associations on galvanic skin responses (GSRs). In these studies they would repeatedly read out a list of neutral words to participants that had to verbalize whatever associated word came to mind. The researchers observed that participants gave different verbal responses to some of the same words and, importantly, that the GSRs were larger than what they had seen before. Notably, this was one of the first psychophysiological experiments and not much was known about the electrodermal response at the time. An in-depth interview with the participants on these words revealed personal affective associations and that the changes in verbal responses had been unintentional. It was concluded that the GSR was able to detect affective associations with neutral words. The different verbal responses and GSRs together were assumed to be a new method to measure an attempt of the mind to prohibit further conscious processing of something that was considered harmful to the self and was referred to as the *psycho-physical galvanic reflex*. Although the authors faced considerable methodological restrictions using the electrodermal response, it seems that these findings are the first (published) displays of the physiological changes that involuntarily accompany an affective state. Later, McGinnies (1949, 110) was able to display negative affective words below threshold of awareness using a tachistoscope at an interval of 10 ms. He found larger GSRs to the affective words compared to the neutral words, which was interpreted as evidence for *perceptual defense*: a distortion of perception to protect the individual from unpleasant experiences. Moreover, Lazarus and McCleary (1951, 111) provided evidence that after a conditioning procedure individuals were able to discriminate between stimuli of different affective valence before conscious recognition as indicated with changes in GSR, which was referred to as *subception*. Notably, the results of these studies have been largely discussed in light of the *repression hypothesis* as they were believed to indicate that individuals tend to reject and keep something out of consciousness when it may negatively affect one's wellbeing. These experimental researchers were pioneers and gave way to find ostensibly more objective evidence of physiological effects of subliminal negative affective information. The research instigated fierce criticism from peers, who performed what we would now call observational studies, and, as a result of the zeitgeist, may have been overlooked in their importance (for a historical discussion the reader is referred to MacKinnon and Dukes, 1962, 48).

More recently, influential evidence of the effects of subliminally presented negative affective stimuli on physiology is offered by neuroscience studies that have found amygdala activation in response to fear-inducing stimuli that were presented below threshold of awareness (e.g., 41,45,46). These findings suggest physiological arousal can be elicited using this type of stimulus presentation and support the earlier findings with GSR that differences in affective valence of stimuli can be determined even when these are presented outside of awareness. However, far less studies seem to have addressed peripheral physiological parameters, such as blood pressure or cortisol. Considering the potential relevance of unconscious processes in psychosomatic research, the aim of the current study was to provide a systematic review of the evidence for the physiological effects of subliminally presented negative affective stimuli from different fields within psychology.

This systematic review focused on studies that manipulated awareness of negative affective stimuli. In experimental designs, awareness is usually manipulated by presenting a stimulus below the threshold of awareness (i.e., subliminally) typically followed (and often preceded) by an irrelevant different stimulus (i.e., mask) (e.g., 51,112-114). Typically, this subliminal manipulation has been applied to two paradigms: priming with stimuli with an innate affective valence (e.g., 67), from here on referred to as 'priming studies', and priming with fear conditioned stimuli (e.g., 51), from here on referred to as 'fear conditioning studies'. The mechanism underlying the first paradigm, priming, is believed to be the activation of cognitive representations of the prime content, which is reflected in a change in a variety of behavioral responses such as reaction times to targets (66). In addition to behavioral responses, physiological responses have also been found to be influenced by subliminal affective primes (e.g., 62). In fear conditioning, an association between an unconditioned stimulus (US), such as a shock or a loud noise, that automatically elicits a response (i.e., unconditioned conditioned response, UCS) and a novel stimulus is formed. The result is a conditioned response (CR) to the now conditioned stimulus (CS+). In contrast, the stimuli that are not combined with a US are referred to as CS-. The participant is assumed to learn to differentiate between the CS+ and CS-. Presentation of the CS+ is expected to elicit a physiological response that is similar to presentation of the US alone, as if it was the negative experience itself (e.g., 68). The advantage of fear conditioning over priming is that it offers more control over the specific affective associations with the stimulus.

Theoretically, the subliminal presentation of negative affective stimuli in experimental paradigms activates unconscious negative affectivity and should result in measurable changes in physiological activity (26-28). Since the dysregulation of adaptive peripheral physiological activity is assumed to be the final step in the relation between psychological negative affect and adverse health outcomes (e.g., 115), we only included studies using peripheral physiological parameters. Most of these parameters are believed to be more directly involved in increased somatic health risks than central nervous system parameters. For example stronger responses of systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate variability (HRV) to mental stress were found to be predictive of cardiovascular (CV) disease risk and other health-related outcomes (e.g., 2,116,117). Furthermore, chronically elevated cortisol increases vulnerability for disease states, for example through immunosuppression and numerous other pathophysiological effects (118). As described, results generally confirm that subliminally presented stimuli affect the brain (e.g., 41,45,46), but this central activity does not necessarily provide information on peripheral activity. Moreover, findings regarding central activity have already been substantially elaborated on elsewhere (e.g., 40,119). In contrast, results on peripheral activity have scarcely been addressed and the potential health risks have not been evaluated. Thus, we focused on the peripheral physiological parameters that indicate physiological changes within the organism: CV and electrodermal (EDA) parameters of autonomic activity, musculoskeletal (i.e., electromyographical; EMG), hormonal, and immunological parameters. Additionally, by including only studies that tested a healthy population we attempted to elucidate the more general mechanisms that theoretically precede physical illnesses.

Searching the literature for research on the main concepts of this study, such as 'unconscious', is considerably hindered by a lack of consensus on terminology (see also 27,120,121). To overcome this issue we paid special attention to building a comprehensive keyword profile in an attempt to find all relevant studies. The complex method of building this profile is explained in detail in the method section. Basically, we systematically expanded an initial simple keyword profile with a large set of new keywords. Possible relevant keywords for 'unconscious' were for example alternatives such as 'subconscious' and 'without awareness'. A comprehensive and systematically built topic-specific profile increases the degree of certainty in finding all relevant articles. Moreover, it ensures replicability across databases and researchers while facilitating updates with exactly the same search profile over time.

Furthermore, we addressed two methodological issues regarding subliminal stimulus presentation. First, as pointed out by Eriksen (1960, 120) and Merikle (1984, 121), to obtain valid results regarding the effects of subliminally presented stimuli, a check of awareness of the presented stimuli is required to ensure that the stimuli are indeed not consciously perceived. Moreover, verbal report of awareness is subjective and objective measures of (non)awareness should be used (121). However, when recognition is reported using an objective measure, it implies that a participant has also consciously perceived (or processed) the stimulus, which is not necessarily true (122). To overcome this conundrum, we have extracted information on the type of awareness check without ascribing any value to the specific type of check. Second, changes in physiology after subliminal presentation of stimuli may be a consequence of the procedure itself, for example by seeing flashes on the screen or the use of masks that might have been arousing in some way. We addressed this by selecting studies with adequate control stimuli (i.e., stimuli that had no negative affective connotation) that were presented in the same way as the negative affective stimulus, either in between-groups or within-group designs.

Taken together, the primary research question of this systematic review is whether subliminally presented negative affective stimuli increase peripheral physiological activity compared with control stimuli. By providing an overview of studies regarding the role of nonconscious processes and potentially pathophysiologic mechanisms, this systematic review may add significant overarching knowledge about the effect of negative affective information on somatic health.

Methods

Keyword profile

We composed an elaborative keyword profile using BOOLEAN logic to formulate and combine the three sets of keywords pertaining to the three concepts: “unconscious”, “negative affect”, and “physiology”. We started with a basic keyword profile in which the sets were separated by ‘AND’: *(unconscious* OR subconscious* OR nonconscious OR non-conscious OR preconscious OR pre-conscious OR sublimin* OR implicit*) AND (stress* OR arousal* OR (negative and (affect* OR emot*)) OR anxi* OR anger OR angr* OR fear OR threat*) AND (cortis* OR glucocort* OR adren* OR noradren* OR SCL* OR GSR* OR blood* OR blood-pressure OR systol* OR diastol* OR cardiac* OR heart* OR cardiovasc* OR immun*).* Subsequently, for each set we aimed to gather an exhaustive list of alternative keywords through the help of a native English speaker, the Thesaurus of PsycINFO, the synonym list of MS Word 2010, and previously found articles. For example in the case of the set “unconscious” we came up with 64 different conceptualizations, such as “nonconscious”, “proprioception”, and “repressed”, see Table 1. Some keywords were written differently across the articles and were thus formulated in all possible ways, for example "mindwandering", "mind-wandering", and "mind wandering". Instead of adding all keywords at once to the basic keyword profile each new keyword was added individually and its additional value was evaluated in terms of the number of new relevant articles found. This was established by searching the databases with a profile containing the new word and the two sets to which the word did not belong, while the set to which the new word did belong was "excluded" by using the NOT function of BOOLEAN logic. For instance in the case of the word "repressed" the evaluative profile would be: *repressed AND (*set keywords for "stress"*) AND (*set keywords for "physiology"*) NOT (*set keywords for "unconscious" without the new keyword*)*. This profile would yield *only* the articles that the keyword "repressed" added to the basic profile. When these articles were considered to be relevant, the keyword was added to its set in the basic profile. When the new keyword did not yield relevant articles it was not used anymore. The final profile that was build using this procedure is provided in Table 2.

TABLE 1 Keywords for "unconscious"

absence of awareness	latent inhibition	repressed
absent-minded	less conscious	represser
access dissociation	masked	repressing
affective stimuli	masked pictures	routinized
affective valence	masked stimuli	stimulus awareness
automatic processing	meta-consciousness	subconscious
automatic emotional	mind-wandering	subliminal
aware	non verbal	suboptimal
awareness	nonattended	suppressed
conscious awareness	nonconscious	suppresser
daydreaming	oblivious	suppressing
degree of awareness	outside of awareness	train of thought
emotional awareness	preattented	unaware
first order mental states	preattentive	unawareness
habitual	preconscious	unconscious
implicit	pre-cognition	unknowing
interoceptive awareness	precognitive	unnoticed unwanted thoughts
intuition	primary proces-level	unpremeditated
intuitive	prime	unwitting
involuntary	priming	without attention
lack of attention	proprioception	
latent	proprioceptive	

TABLE 2 Keyword profiles as inserted into the databases

Database	Web of Science	PsycINFO
Search details	Core Collection Advanced Search	Basic Search
Keyword profile	((TS=(unconscious* or subconscious* or nonconscious or non-conscious or preconscious or pre-conscious or sublimin* or implicit* or "automatic emotional" or "automatic emotion" or "automatic affect" or "automatic affective" or unattend* or mind-wandering or "emotional awareness" or "interoceptive awareness" or "degree of awareness" or "stimulus awareness" or "conscious awareness" or "involuntary stress" or "latent inhibition" or precogn* or pre-attent* or "automatic processing" or masked* or nonverbal or "non verbal communication") AND TS=(stress* or arousal* or (negative and (affect* or emot*)) or anxi* or anger or angr* or fear or threat* or ruminat* or worr* or "psychological tension" or shock* or "affective stimuli" or "priming" or "prime" or (emotional and (stimuli or circuit* or content* or state* or stimulation or expression))) AND TS= (cortis* or glucocort* or adren* or noradren* or SCL* or GSR* or blood* or blood-pressure or systol* or diastol* or cardiac* or heart* or cardiovasc* or immun* or "physiological arousal" or "physiological measures" or "physiological correlates" or "physiological activity" or "skin conductance" or autonomic* or EMG or (fac* AND (electromyography or muscle*))))) *AND* **LANGUAGE:** (English) *AND* **DOCUMENT TYPES:** (Article)	(unconscious* or subconscious* or nonconscious or non-conscious or preconscious or pre-conscious or sublimin* or implicit* or "automatic emotional" or "automatic emotion" or "automatic affect" or "automatic affective" or unattend* or mind-wandering or "emotional awareness" or "interoceptive awareness" or "degree of awareness" or "stimulus awareness" or "conscious awareness" or "involuntary stress" or "latent inhibition" or precogn* or pre-attent* or "automatic processing" or masked* or nonverbal or "non verbal communication") AND (stress* or arousal* or (negative and (affect* or emot*)) or anxi* or anger or angr* or fear or threat* or ruminat* or worr* or "psychological tension" or shock* or "affective stimuli" or "priming" or "prime" or (emotional and (stimuli or circuit* or content* or state* or stimulation or expression))) AND (cortis* or glucocort* or adren* or noradren* or SCL* or GSR* or blood* or blood-pressure or systol* or diastol* or cardiac* or heart* or cardiovasc* or immun* or "physiological arousal" or "physiological measures" or "physiological correlates" or "physiological activity" or "skin conductance" or autonomic* or EMG or (fac* AND (electromyography or muscle*)))
Limiters	Indexes=SCI-EXPANDED, SSCI Timespan=All years	Peer-reviewed Human subjects

Search strategy

The procedures described by the PRISMA (Preferred reporting Items for Systematic Reviews and Meta-Analyses) Statement (123) were applied, to the extent that they apply to experimental research, to the literature search, data collection, and reporting of the results. The final keyword profile was used in Web of Knowledge (Core collection; field: 'topic') and PsycINFO (field: 'all text') on June 16, 2015. In Web of Science the search was limited to 'Article' as document type and 'English' as language. The used indexes were 'SCI-Expanded' and 'SSCI'. No limit to the time span was applied. In PsycINFO the limiters 'peer-reviewed' and 'human subjects' were applied. All duplicate publications were removed. For seven eligible articles the full-text could not be obtained through online methods; in one case we received the full-text version of the article from the authors, in two cases the authors were already deceased, and in the remaining four cases there was no response from the authors. The latter studies were discarded (124-127). Finally, we checked all references of the final selection of articles (i.e., a snowballing procedure) for articles that might not have been picked up by the keyword-profile. This resulted in ten possible new inclusions, of which three were eligible for inclusion. The databases were checked again for new articles on 16 December 2015 and resulted in one additional relevant article. Finally, one eligible article was accepted for publication at time of the second search and was obtained through personal communication.

Study selection and data collection

In total 2301 articles were evaluated for eligibility (See Figure 1). Articles were included when (1) subjects were healthy human adults, (2) an experimental design was used, (3) manipulation involved a negative affective stimulus, (4) the negative affective stimulus was manipulated out of the subject's awareness, i.e., processed without requiring conscious processing, (5) a control stimulus was used that was presented exactly like the negative affective stimulus for either between or within-group designs but was either of positive or neutral valence, (6) the dependent measure was a peripheral physiological outcome measure, (7) the article was peer-reviewed (e.g., no dissertations, conference proceedings, or editorials), (8) full-text was available in either English or Dutch.[1]

[1] The articles by Jung (1907, 49), Peterson and Jung (1907, 50), and McGinnies (1949, 110) were not included in the review. Although they are relevant in terms of the historical context of this systematic review, they were not found with our search strategy and did not meet the inclusion criteria. First, they were not selected using the keyword profile since the studies did not use a combination of the selected keywords. Furthermore, the articles did not include an abstract. Additionally, the snowballing procedure did not lead to inclusion of these articles. Moreover, the studies by Jung (1907, 49) and Peterson and Jung (1907, 50) did not include negative affective stimuli and the study by McGinnies (1949, 110) used 'critical words' as a manipulation, that would now probably be classified as high arousing rather than negative affective (raped, belly, whore, kotex, penis, filth, bitch). The studies would thus have been excluded for the review.

Eligibility was evaluated independently by two reviewers, the first and third author. A third reviewer, the second author, was consulted in case of disagreement. Articles that could not unanimously be excluded based on the information available at one step automatically were included in the next step to prevent invalid exclusion. The first round of exclusion was based on title; articles with titles that clearly implied an unrelated subject were discarded. After this round 679 articles were left. In the second round, exclusion was based on abstract and resulted in 184 potential eligible articles. Finally, in the third round the full-texts were evaluated which lead to the final inclusion of 54 articles. From articles that discussed multiple experiments studies that met the inclusion criteria were included as separate studies, resulting in a final selection of 65 studies.

The main features of the studies were extracted, as displayed in Table 3: Sample description, the nature of the negative affective stimulus, the key features of the design such as type of stimuli and presentation method, the type and data handling of the physiological parameters, awareness check, and the results. Data extraction was checked by at least one other author.

Quality assessment

To our knowledge no standardized quality assessment of experimental designs in psychology is available. To this end, we combined the Cochrane Collaboration's tool for assessing risk of bias (181) and the CAMARADES checklist (182) for quality of experimental animal studies. This resulted in a novel study quality assessment checklist of 15 items, of which 13 are applicable to experimental designs in psychology in general and two are specific for this systematic review. The items are displayed in Table 4.

The articles were awarded one point for each item, with a minimum score of 1 and maximum score of 15. The criteria were applied to articles and supplementary material. The findings are reported in Table 5 through 7 but are not further incorporated in decisions on eligibility or data collection since the assessment has not been used previously. However, the information was incorporated in the final conclusions regarding the research question.

Reporting

Performing a meta-analysis was not feasible due to the variety of employed stimuli and physiological parameters and differences in measurement procedures within studies using the same parameter (see Table 3). All results that addressed the current research question are reported based on the statistical significance as reported in the articles. Findings are summarized in terms of the specific *contrasts* that tested the effect of the negative affective stimulus compared with the control stimulus on the physiological parameters. The effect sizes were calculated and reported as η^2,η_p^2 or *d*, as appropriate (183). Furthermore, the nature of the awareness checks performed in the studies are reported in Table 3. See Table 5 through 7 for the results per type of physiological parameter and type of experimental manipulation.

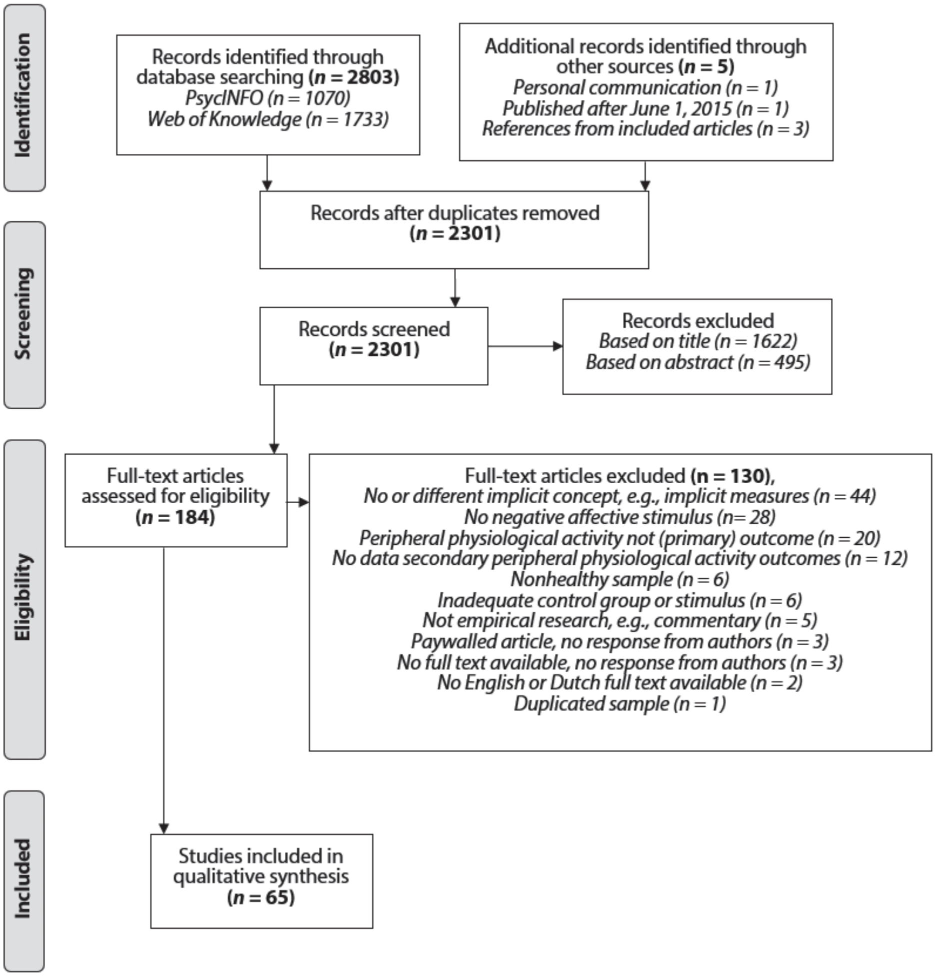


FIGURE 1 Flow chart of the selection process. Adapted from Moher et al. (2009, 123)

TABLE 3 Study descriptions split by type of manipulation (fear conditioning and priming)

Fear conditioning studies					
Author	*Year*	*Sample (age, N)*	*Subliminal manipulation*	*Stimulus characteristics*	*Awareness check*
Aderman et al.	1964	students, 18-25, 11	Subliminal extinction through variation in illumination on a projector	US: Shock after odd numbered stimuli, 75% reinforcement CS: Squares variating in size composed of parallel beams of white light projected onto the back of a milk-glass screen	Verbal report through individual threshold detection prior to study
Beisgen and Gibby	1969	volunteers, 19-25, 17	Subliminal acquisition and extinction through variation in exposure speed (CS: 50 - 200 ms)	US: Shock after CS+, 50% reinforcement CS: Set of nonsense syllables, five selected as CS+	Verbal report through individual threshold detection prior to study
Bunce et al.	1999	Males volunteers, 21.6 (1.4), 8	Subliminal acquisition through fast exposure (CS: 2 ms, CS-US 800 ms SOA)	US: Shock after CS+, 100% reinforcement CS: Schematic unpleasant face (CS+) and pleasant face (CS-)	Forced recognition task and visual threshold technique
Cornwell et al.	2007	volunteers, 18-48, 28	Subliminal acquisition (partial) through dichoptic configured images (CS: 50 ms, mask: 850 ms, on 50 ms, off 150 ms, alternating)	US: Shocks with unmasked presentation of CS+, 50% reinforcement CS: Red or green + or x sign Mask: Opponent colored stimulus	Verbal report
Corteen and Wood	1972	students, 19-34, 24	Dichotic listening in extinction through auditory masking (prose passage in one ear and target in the other)	US: Shocks after CS+ offset, 100% reinforcement CS: City names (CS+) and nouns (CS-) Mask: Prose passage	Verbal report
Flo et al.	2011	volunteers, 20-40, 16	Acquisition and extinction during S2 and REM sleep stages	US: Shocks or aversive images presented simultaneously with CS+, 100% reinforcement CS: Neutral sounds	N.A. (extinction during sleep)
Flykt et al. (study 1)	2007	students, 19-40, 64	Subliminal extinction through fast exposure and backward masking (CS: 25 ms, mask: 125 ms)	US: Shock or broad-band noise burst after CS+, 87.5% reinforcement CS: Snakes/guns away/towards the viewer Mask: Scrambled different images	Forced recognition task and verbal report
Flykt et al. (study 2)	2007	students, 18-44, 32	Subliminal extinction through fast exposure and backward masking (CS: 30 ms, mask: 175 ms)	US: Shock or broad-band noise burst after CS+, 81.3% reinforcement CS: Snakes/guns away/towards subject Mask: Scrambled different images	Forced recognition task and verbal report

Author	Year	Sample (age,N)	Subliminal manipulation	Stimulus characteristics	Awareness check
Golkar and Öhman	2012	students, 24.9 (5.3), 27	Subliminal extinction through fast exposure and backward masking (CS: 33 ms, mask: 6 s)	US: Shock during CS+ 100%reinforcement CS: Fearful face images Mask: Neutral face images Other: Acoustic startle probe at six of the CSs	Forced recognition task
Lazarus and McCleary	1951	n.r., n.r., 9	Subliminal extinction through variation in 5 exposure times (CS: from 1/150 s to 1 s)	US: Shock after CS+ offset, 33.3% reinforcement CS: Nonsense syllables	Verbal report through individual threshold detection
Lipp et al.	2014	volunteers, 20.5 (2.44), 30	Binocular switch suppression through alternating the target and mask between visual fields with the target changing from black to full brightness at a rate of 2 Hz	US: Shock after CS+, 100% reinforcement CS: Image of snakes or wallabies Mask: Colored noise image	Signal detection experiment and verbal report
Núñez and De Vicente (study 1)	2004	students, 17-28, 36	Subliminal acquisition through fast exposure and backward masking (CS: individually determined, mask: 50 ms)	US: Shock after CS+, 100% reinforcement CS: The words 'magnesio' or 'locuacidad' Mask: XOXOXOXOXO	Individual threshold detection
Núñez and De Vicente (study 2)	2004	students, 17-25, 24	Subliminal acquisition through fast exposure and backward masking (CS: individually determined, mask: 50 ms)	US: Shock after CS+, 100% reinforcement CS: Two neutral words, two nonwords Mask: XOXOXOXOXO	Individual threshold detection
Öhman and Soares (study 2)	1993	students, 18-47, 64	Subliminal extinction through fast exposure and backward masking (CS: 30 ms, mask: 100 ms)	US: Shock after CS+, 83.3% reinforcement CS: Fear-relevant (spider, snake), fear-irrelevant (flower, mushroom) images Mask: Scrambled different images	Forced recognition task
Öhman and Soares (study 3)	1993	students, 17-47, 32	Subliminal extinction through fast exposure and backward masking in alternating visual fields (CS: 30 ms, mask: 100 ms)	US: Shock after CS+, 83.3% reinforcement CS: Fear-relevant (spider, snake), fear-irrelevant (flower, mushroom) images Mask: Scrambled different images	Forced recognition task
Öhman and Soares (study 1)	1998	students, 24.1 (n.r.), 40	Subliminal acquisition through fast exposure and backward masking (CS: 30 ms, mask: 100 ms)	US: Shock after CS+, 83.3% reinforcement CS: Fear-relevant or irrelevant images Mask: Scrambled different images	n.r.
Öhman and Soares (study 2)	1998	students, 19-39, 48	Subliminal acquisition and extinction through fast exposure and backward masking (CS: 30 ms, mask: 100 ms)	US: Shock after CS+ onset, 100% reinforced CS: Fear-relevant images Mask: Scrambled different images	Forced recognition task

Author	*Year*	*Sample (age,N)*	*Subliminal manipulation*	*Stimulus characteristics*	*Awareness check*
Olsson and Phelps	2004	students, n.r., 87	Subliminal acquisition and extinction through fast exposure and backward masking (CS: 33 ms, mask: 5973 ms)	US: Shock during CS+, unmasked trials only CS: Two angry male face images Mask: Neutral male face image	Verbal report
Parra et al. (study 1)	1997	students, 22.1 (n.r.), 24	Subliminal extinction through fast exposure and backward masking (CS: 30 ms, mask: 30 ms)	US: Shock after CS+, 94,4% reinforcement CS: Angry, happy, and neutral male face images Mask: Face with similar valence but different from CS	Familiarity test
Parra et al. (study 2)	1997	students, 22.7 (n.r.), 40	Subliminal acquisition and extinction through fast exposure and backward masking (CS : 30 ms, mask: 30 ms)	US: Shock after CS+, 83.3% reinforcement CS: Angry or happy male face images Mask: Neutral face images	Familiarity test
Peper and Karcher	2001	volunteers, Group 1: 24.7 (5), 22	Subliminal acquisition and extinction through backward masking (CS: 30 ms, masks: in total 500 ms)	US: Baby cry (96 dB) after CS+, 71.4 % reinforcement CS: Face images without visual features but with positive or negative valence Mask: Scrambled facial elements	Forced recognition task
Saban and Hugdahl	1999	students, 18-28, 24	Subliminal extinction through fast exposure and backward masking (CS: 30 ms, mask: 100 ms)	US: Noise (90 dB) after CS+, 100% reinforcement CS: Two different male angry face images Mask: Neutral male face.	n.r.
Soares and Öhman	1993	volunteers, 18-47, 128	Subliminal extinction through backward masking (CS: 30 ms, mask: 100 ms)	US: Shocks during CS+, 83.3% reinforcement CS: Two fear-relevant or two neutral images Mask: Scrambled CS images	n.r.
Tassinary et al.	1984	students, n.r., 24	Subliminal extinction through fast exposure and dichoptic backward masking (CS: 10 ms nondominant eye, mask: 20 ms in dominant eye and 500 ms in both eyes)	US: Shock after CS+, 87.5% reinforcement CS: Names of body parts and animals Mask: Pattern mask (i.e., superimposed uppercase letters) Task: Lexical Decision Task[1]	n.r.
Wall and Guthrie	1959	students, n.r., 10	Subliminal extinction (partial) through fast exposure (CS: 1 s)	US: Shocks during CS+, 100% reinforcement CS: Neutral words Other: In testphase 1 CS visible in 50% of the trials	Verbal report
Wardlaw and Kroll	1976	n.r., n.r., 18	Dichotic listening in extinction through auditory masking (prose passage in one ear and target in the other)	US: Shocks after CS+, 100% reinforcement CS: City names (CS+) and nouns (CS-) Mask: Prose passage	Verbal report
Wiens et al.	2003	students, 18-28, 85	Subliminal acquisition through fast exposure and backward masking (CS: 10 ms, mask: 50 ms)	US: Shock after mask, group dependent reinforcement CS: Spider and snake images Mask: Scrambled CS images	Forced recognition task

Author	*Year*	*Sample (age,N)*	*Subliminal manipulation*	*Stimulus characteristics*	*Awareness check*
Wong et al.	1994	volunteers, 20.8 (1.4), 17	Subliminal extinction through energy masking with individually determined threshold	US: Shock after CS+, 83.3% reinforcement CS: Pleasant (CS-) and unpleasant (CS+) facial schematics Mask: Bright field with higher energy content than CS	Individual threshold detection
Worthington (study 1)	1966	students, n.r., 16	Subliminal acquisition through high illumination on a projector	US: Shock during CS+, 66.7% reinforcement CS: Words	Individual threshold detection and verbal report
Worthington (study 2)	1966	students, n.r., 16	Subliminal acquisition through high illumination on a projector	US: Shock during CS+, 66.7% reinforcement CS: Words	Individual threshold detection and verbal report
Priming studies					
Author	*Year*	*Sample (age,N)*	*Subliminal manipulation*	*Stimulus characteristics*	*Awareness check*
Bornemann et al.	2012	students, 19.8 (1.39), 57	Subliminal exposure through fast exposure and backward masking (prime: 10/20 ms, mask: 2s)	NA prime: Angry face image Control primes: Happy and neutral face images Mask: Neutral face image or dotted pattern Task: Indicate valence of prime	Forced recognition task
Chatelain and Gendolla (study 1)	2015	students, 25, n.r., 42	Subliminal exposure through fast exposure and backward masking (prime: 27 ms, mask: 133 ms)	NA primes: Angry or fearful faces, 1/3 of the trials[2] Control prime: Happy faces, 1/3 of the trials Mask: Noise picture of black/white dots Task: Parity task[3]	Funneled debriefing
Codispoti et al. (study 1)	2009	students, n.r., > 42	Subliminal exposure through fast exposure (target: 25 and 80 ms)	NA prime: Unpleasant images Control primes: Pleasant and neutral images Task: None	n.r.
Codispoti et al. (study 2)	2009	students, n.r., 97	Subliminal exposure through fast exposure and backward masking (prime: 25, 40, 50 and 80 ms, mask: 1 s)	NA prime: Unpleasant images Control primes: Pleasant and neutral images Mask: Pattern mask Task: None	n.r.
Dimberg et al.	2000	students, n.r., 120	Subliminal exposure through fast exposure and backward masking (prime: 30 ms, mask: 5 s)	NA prime: Angry face image Control primes: Happy and neutral face images Mask: Neutral face image Task: None	Pilot study and verbal report

Author	*Year*	*Sample (age,N)*	*Subliminal manipulation*	*Stimulus characteristics*	*Awareness check*
Garfinkel et al. (study 1)	2016	students, 22.7 (1.1), 18	Subliminal exposure through fast exposure and sandwich masking (forward mask: 17 ms, prime: 17 ms, backward mask: 50 ms)	NA prime: The word 'anger' Control prime: The word 'relax' Mask: String of letters Task: Lexical Decision Task[1]	n.r.
Garfinkel et al. (study 2)	2016	students, 24.6 (5.0), 14	Subliminal exposure through fast exposure and sandwich masking (forward mask: 17 ms, prime: 17 ms, backward mask: 50 ms)	NA prime: The word 'anger' Control prime: The word 'relax' Mask: String of letters Task: Lexical Decision Task[1]	Forced recognition task
Gendolla and Silvestrini (study 1)	2011	students, 22 (n.r.), 45	Subliminal exposure through fast exposure and backward masking (prime: 26 ms, mask: 125 ms)	NA prime: Angry or sad face image, 1/3 of the trials[2] Control prime: Happy face image Mask: N.r. Task: Modified d2 mental concentration task[4]	Forced recognition task
Gendolla and Silvestrini (study 2)	2011	students, 21 (n.r.), 42	Subliminal exposure through fast exposure and backward masking (prime: 26 ms, mask: 125 ms)	NA prime: Angry or sad face image, 1/3 of the trials[2] Control prime: Happy face image Mask: N.r. Task: Modified d2 mental concentration task[4]	Forced recognition task
Hull et al. (study 3)	2002	students, n.r., 33	Subliminal exposure through fast exposure and sandwich masking (forward mask: 17 ms, prime: 17 ms, backward mask: 50 ms)	NA prime: The word 'anger' Control prime: The word 'relax' Mask: String of letters Task: Lexical Decision Task[1]	Verbal report
Hull et al. (study 4)	2002	students, n.r., 64	Subliminal exposure through fast exposure and sandwich masking (forward mask: 17 ms, prime: 17 ms, backward mask: 50 ms)	NA prime: The word 'anger' Control prime: The word 'relax' Mask: String of letters Task: Lexical Decision Task[1]	Verbal report
Jönsson and Sonnby-Borgström	2003	volunteers, 19-35, 53	Subliminal exposure through fast exposure and backward masking (prime: 17 and 56 ms, mask: 63 ms after prime offset)	NA prime: Angry male face images Control prime: Happy male face images Mask: Scrambled neutral face image Task: None	Written report
Kemp-Wheeler and Hill	1987	volunteers, G1: 19.7 (1.39); G2: 18-24, 28	Subliminal exposure through fast exposure and backward masking using dichoptic presentation	NA prime: Negative words Control words: Neutral words Mask: Pattern mask of scrambled numbers Task: None	Verbal report through individual threshold detection before experiment

Author	*Year*	*Sample (age,N)*	*Subliminal manipulation*	*Stimulus characteristics*	*Awareness check*
Kimura et al.	2004	male volunteers, 31.6 (6.4), 11	Subliminal exposure through fast exposure and dichoptic masking (prime: 30 ms, mask: 60 ms)	NA prime: Negative images Control prime: Neutral images Mask: Scramled prime images during prime presentation and flower images after prime presentation Task: Count amount of upside down flower images	Verbal report
Lapate et al.	2014	students, n.r., 46	Dichoptic presentation with emotional stimuli in the nondominant eye (1,023 ms) and mask in dominant eye (1,488 ms)	NA prime: Fearful face and spider images Control prime: Neutral face images Mask: Low contrast versions of prime Task: Indicate extend of liking the person based on a novel face	Forced recognition task and verbal report
Lasauskaite et al.	2013	female students, 20.5 (n.r.), 52	Subliminal exposure through fast exposure and backward masking (prime: 26 ms, mask: 130 ms)	NA prime: Sad face image, 1/3 of the trials[2] Control prime: Happy face images Mask: Scattered black/white dots Task: Modified d2 mental concentration task[4]	None
Lasauskaite Schüpbach et al.	2014	students, 21 (n.r.), 134	Subliminal exposure through fast exposure and backward masking (prime: 27 ms, mask: 133 ms)	NA prime: Sad face image, 1/3 of the trials[2] Control prime: Happy face images Mask: Scattered black/white dots Task: Indicate correctness of an equation	n.r.
Lee and Tyrer	1981	students, 20-28, 48	Subliminal exposure through a neutral density filter of different transmission value in front of a projector lens and superimposed masking field	NA prime: Anxiety inducing motion picture Control prime: Neutral motion picture and motion picture with various abstract displays at two rates (18 ft/s and 24 ft/s) Mask: Neutral density filters reduced image intensity Task: None	Pilot study in different sample and verbal report
Najström and Jansson	2007	police recruits, 27.6 (2.88), 73	Subliminal exposure through fast exposure and backward masking (prime: 6 ms, mask: 200 ms)	NA prime: Unpleasant and arousing images (mostly physical threat) Control prime: Pleasant and calm images Mask: Scrambled prime image Task: None	Threshold detection task prior to study

Author	*Year*	*Sample (age,N)*	*Subliminal manipulation*	*Stimulus characteristics*	*Awareness check*
Nielsen and Kaszniak[5]	2006	paid volunteers, 21.2 (4.73), 17	Subliminal exposure through fast exposure and sandwich masking (forward mask: 45 ms, prime: 45 ms, backward mask: 2910 ms)	NA prime: Unpleasant and arousing images Control prime: Pleasant arousing images and neutral low arousing images Mask: Reassembled prime images Task: None	Forced recognition task
Ravaja et al.	2004	students, 19-35, 33-39	Subliminal exposure through six embedded facial expressions in a video clip (prime: 20 ms, mask: continuous)	NA prime: Angry face image Control primes: Happy or neutral face images Targets: Video clips of news items with different valence and arousal levels Mask: Face of newscaster Task: None	Verbal report
Reagh and Knight	2013	students, 18-24, 19	Subliminal exposure through fast exposure and backward masking (prime: 17 ms, mask: up to 8 s)	NA prime: Negative images Control primes: Positive and neutral images Mask: Random multicolored triangles Other: Startle probe white noise (100 dB) for 500 ms at 2, 4, and 6 s after trial onset during the mask on 72 trials Task: None	Forced recognition task and verbal report
Rotteveel et al. (study 2)	2001	students, 23 (5.9), 40	Subliminal exposure through fast exposure and backward masking (prime: 15 ms, mask: 2000 ms)	NA prime: Angry face images Control primes: Happy and neutral face images Mask: Chinese ideographs and neutral images Task: Indicate gender or valence of mask	Forced recognition task
Ruiz-Padial et al.	2011	female students, 20.4 (2.13), 35	Subliminal exposure through fast exposure and backward masking (prime: 30 ms, mask: 100 ms)	NA prime: Unpleasant images Control primes: Neutral and pleasant images Mask: Unidentifiable display Other: Startle probe noise burst (105 dB) for 50 ms, between 3-4 s after stimulus onset in part of the trials Task: None	Forced recognition task and verbal report
Silvert et al.	2004	female students, 20-27, 17	Subliminal exposure through fast exposure and backward masking (prime: 29.4-47.1 ms, mask: 150 ms)	NA prime: Negative words Control primes: Neutral words Mask: Sequence of #s Task: None	Individual threshold detection

Author	*Year*	*Sample (age,N)*	*Subliminal manipulation*	*Stimulus characteristics*	*Awareness check*
Silvestrini and Gendolla	2011a	students, 23, n.r., 56	Subliminal exposure through fast exposure and backward masking (prime: 26 ms, mask: 125 ms)	NA prime: Sad face image, 1/3 of the trials[2] Control prime: Happy face image Mask: N.r. Task: Modified d2 mental concentration task[4]	Forced recognition test
Silvestrini and Gendolla	2011b	students, 23 (n.r.), 75	Subliminal exposure through fast exposure and backward masking (prime: 26 ms, mask: 125 ms)	NA prime: Sad face image, 1/3, 2/3, or 3/3 of the trials[2] Control prime: Happy face image Mask: N.r. Task: Modified d2 mental concentration task[4]	Forced recognition test
Smith	1993	students, n.r., 39	Subliminal exposure through editing a single frame of the prime into the video (prime: 16.7 ms, mask: continuous)	NA prime: Negative stimuli Control prime: Positive or neutral stimuli Mask: Neutral video Task: None	Verbal report
Sonnby-Borg-ström et al.	2003	students, 19-35, 61	Subliminal exposure through fast exposure and backward masking (prime: 17 and 53 ms, mask: 63 ms)	NA prime: Angry face image Control prime: Happy face image Mask: Nonfigurative grey-scale picture Task: None	n.r.
Sonnby-Borg-ström et al.	2008	volunteers, median 24, 100	Subliminal exposure through fast exposure and backward masking (prime: 17 - 23 or 65 - 70 ms, mask: 100 ms)	NA prime: Angry and sad face image Control prime: Happy face image Mask: N.r. Task: None	Pilot study and verbal report
Tan et al. (study 3)	2013	students, 21.6 (1.69), 36	Subliminal exposure through fast exposure and sandwich masking (forward mask: 133 ms, prime: 40 ms, backward mask: 133 ms)	NA prime: Negative images of animals or objects Control primes: Neutral images of animals or objects Mask: Abstract images Task: Respond to square	Forced recognition task
Weisbuch-Remington et al. (study 1)	2005	students, n.r., 107	Subliminal exposure through fast exposure and backward masking (prime: 30 ms, mask: 1000 ms)	NA prime: Negative religious images Control prime: Positive religious images Mask: Same image reassembled and 180° rotated Task: Count number of tiles on mask	Verbal report

Author	*Year*	*Sample (age,N)*	*Subliminal manipulation*	*Stimulus characteristics*	*Awareness check*
Weisbuch-Remington et al. (study 2)	2005	students, n.r., 191	Subliminal exposure through fast exposure and backward masking (prime: 30 ms, mask: 1000 ms)	NA prime: Negative religious and nonreligious images Control prime: Positive religious images and blurred religious images, 180° rotated Mask: Same image reassembled and 180° rotated Task: Count number of tiles on mask	Verbal report
Williams et al.	2006	volunteers, 35.8 (9.06), 15	Subliminal exposure through fast exposure and backward masking (prime: 16.7 ms, mask: 150 ms)	NA prime: Fear face image Control prime: Neutral face image Mask: Neutral face image Task: None	Forced recognition task and individual threshold detection
Williams et al.	2004	volunteers, 24.9 (7.5), 20	Subliminal exposure through fast exposure and backward masking (SOA: 10 ms and 30 ms, mask: 100 ms)	NA prime: Fear face image Control prime: Neutral face image Mask: Neutral face image Task: Indicate gender or age category of mask	Individual threshold detection

Note. The displayed sample descriptions are: the nature of the sample, age (*M* (*SD*) or range) and *N* (analyzed). *Abbreviations*: US = Unconditioned stimulus, CS = Conditioned stimulus, CS+ = CS paired with US, CS- = CS not paired with US, ms = Milliseconds, SOA = Stimulus onset asynchrony, NA = Negative affective, n.r. = Not reported, N.A. = Not available, S2 = Stage 2 (sleep stage) REM = Rapid eye movement (sleep stage), s = Seconds, Hz = Hertz, dB = Decibel, G = Group, ft = Feet.

[1] In a Lexical Decision Task letter strings, or targets, are presented that form words or nonwords that have to be categorized accordingly. The primes precede these strings (e.g., 67).

[2] In the remainder of the trials a neutral face image was used as a prime.

[3] Parity task: Paradigm to test attentional engagement to emotional stimuli (179).

[4] In this study participants had to discriminate between two or other amount of apostrophes around p or d, which is a modified version from the original d2 test of attention (180).

[5] The study compares meditators and controls, but for the current review the data for the healthy controls were extracted

TABLE 4 Description of the criteria used for the quality assessment of the studies

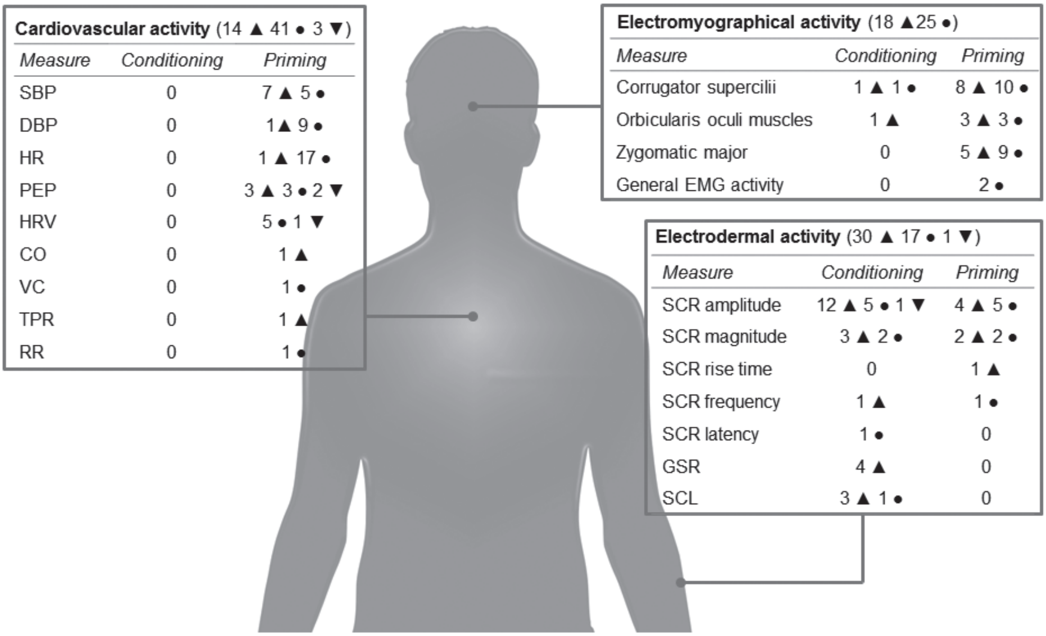
Criterion	Description
1.	Publication in peer-reviewed journal
2.	Complete sample description (indication of age and nature of sample)
3.	Reports sample size and number of drop-outs
4.	Randomized allocation of participants to groups in between-subjects designs and randomized presentation of stimuli across trials in within-subject designs
5.	Inclusion of a control group in between-subjects designs, or control trials or phases in within-subject designs
6.	Blinded researcher
7	Blinded allocation or outcome concealment
8.	Ethical aspects addressed by a review committee
9.	Report Mean and Standard Deviation or Standard Error of the Mean and/or effect size
10.	Report outcomes of statistical tests (*F*/*T* ratios or regression coefficients)
11.	Report significant and nonsignificant results
12.	Report outcomes of all dependent variables described in method section
13.	Statement on possible conflict of interest
14.	Included an awareness check
15.	Description of method of physiological data handling

Results

Nature of the included studies

A summary of the results can be found in Figure 2. Importantly, the literature search did not reveal any studies with hormonal or immunological parameters and results are thus restricted to the CV, EDA, and EMG parameters. Furthermore, the search was not limited to specific experimental methods, but only articles using a fear conditioning or priming paradigm were found. Finally, the study samples usually consisted of students, but in several studies other groups were included such as unpaid and paid nonstudent volunteers, police recruits (149), and meditators versus nonmeditators (150).

Fear conditioning studies. Thirty studies used fear conditioning to induce a negative affective state. These experiments consist of an acquisition phase, during which the CR is created, and an extinction phase, during which the occurrence of the CR in absence of the US is observed (e.g., 68). In both phases stimuli can be presented subliminally. The CR to a CS+ is compared with responses to the CS-. CRs were only considered, in both the acquisition and extinction phase, when the US was not presented or presented outside the time-window during which the CR was measured. The CS+ usually consisted of fear-relevant images (e.g., spiders), or neutral stimuli (e.g., neutral shapes or sounds).



Cardiovascular activity (14 ▲ 41 ● 3 ▼)		
Measure	*Conditioning*	*Priming*
SBP	0	7 ▲ 5 ●
DBP	0	1▲ 9 ●
HR	0	1 ▲ 17 ●
PEP	0	3 ▲ 3 ● 2 ▼
HRV	0	5 ● 1 ▼
CO	0	1 ▲
VC	0	1 ●
TPR	0	1 ▲
RR	0	1 ●

Electromyographical activity (18 ▲25 ●)		
Measure	*Conditioning*	*Priming*
Corrugator supercilii	1 ▲ 1 ●	8 ▲ 10 ●
Orbicularis oculi muscles	1 ▲	3 ▲ 3 ●
Zygomatic major	0	5 ▲ 9 ●
General EMG activity	0	2 ●

Electrodermal activity (30 ▲ 17 ● 1 ▼)		
Measure	*Conditioning*	*Priming*
SCR amplitude	12 ▲ 5 ● 1 ▼	4 ▲ 5 ●
SCR magnitude	3 ▲ 2 ●	2 ▲ 2 ●
SCR rise time	0	1 ▲
SCR frequency	1 ▲	1 ●
SCR latency	1 ●	0
GSR	4 ▲	0
SCL	3 ▲ 1 ●	0

FIGURE 2 Schematic summary of the results expressed in the number of tested contrasts for each type of outcome measure per type of study. The direction of the results are indicated with ▲(NA stimuli > control stimuli), ▼(NA stimuli < control stimuli), and ● (no difference between the stimulus types). *Abbreviations*: SBP = Systolic blood pressure, DBP = Diastolic blood pressure, HR = Heart rate, PEP = Pre-ejection period, HRV = Heart rate variability, CO = Cardiac output, VC = Ventricular contractility, TPR = Total peripheral resistance, RR = Respiratory rate, GSR = Galvanic skin response, SCR = Skin conductance response, SCL = Skin conductance level

Subliminal presentation was achieved through different procedures: fast presentation with and without backward masking, brightness differences (i.e., illumination), dichoptic presentation (i.e., presentation of different stimuli to each eye), and dichotic listening (i.e., presentation of different stimuli to each ear). Less common techniques to induce the CRs involved procedures during sleep, binocular switch suppression (i.e., intermittently switching the stimulus and mask between both eyes), and energy masking (i.e., extremely limited stimulus exposure before a blank field of equal luminance). As awareness check, the studies generally used verbal report, but several studies used more objective methods such as a threshold detection task and forced recognition of the stimuli. Some studies did not report an awareness check (13.3%) and in one study this was deemed unnecessary since the participants were asleep during the experiment.

Priming studies. Thirty-five studies used subliminal priming to induce a negative affective state. In these experiments affective stimuli, referred to as 'primes', are presented during an often irrelevant task. Responses are compared with those to positive or neutral (i.e., control) stimuli. In most studies, the negative affective primes were images of faces displaying a negative emotion (anger, sadness, or fear), but also other unpleasant images, negative words, or an anxiety inducing motion picture.

In most priming studies, subliminal presentation was achieved through fast presentation with backward masking, 'sandwich masking' (i.e., presenting a mask before and after the prime), or fast exposure without masking. Some studies embedded the stimuli into a video clip, used dichoptic presentation, or placed a neutral density filter in front of a projector lens. Generally, verbal report was used to check whether participants had been aware of the stimuli. Other methods were a pilot study, individual threshold detection tasks, forced recognition tasks, or a funneled debriefing. Not all studies (15.2%) reported an awareness check and one study reported that an awareness check was not performed.

Findings of the included studies

Electrodermal activity (EDA). EDA measures are based on several different quantifications of skin conductance recordings (for a description of the specific measures the reader is referred to 103). The findings are displayed in Table 5 and operationalizations of the EDA measure are reported for each study.

Fear conditioning studies. In 23 fear conditioning studies, EDA responses were measured to test 33 contrasts that compared subliminally presented negative affective stimuli (CS+) with control stimuli (CS-). In 12 out of the 18 contrasts measuring *skin conductance response* (SCR) *amplitude* a larger response to the CS+ compared to the CS- was found, while five contrasts yielded no difference between the CS+ and CS-. Furthermore, one study found a reversed effect, that is, the responses to the CS+ were smaller compared with the CS-. Several other EDA measures were used; *SCR magnitude* was higher in three contrasts but lower in two other contrasts, the number of *galvanic skin responses* (GSRs) was larger in four contrasts, changes in *skin conductance level* (SCL) were larger in three contrasts and equal in one contrast, *SCR frequency* was higher in one contrast, and, finally, no changes were found for *SCR latency* as tested by one contrast.

Importantly, a higher SCR amplitude was generally found in response to fear-relevant CS+ as opposed to fear-irrelevant CS+ that elicited a higher SCR amplitude. Regarding the other EDA parameters, most studies used fear-relevant stimuli, but did not compare them to fear-irrelevant stimuli. Only Lipp, Kempnich, Jee, and Arnold (2014, 148) made this comparison, by using snakes (fear-relevant) and wallabies (fear-irrelevant) as conditioned stimuli, and did not find a difference in the responses.

Priming studies. The majority of the 10 priming studies, testing 15 contrasts, measured *SCR amplitude*. Four contrasts showed higher SCR amplitude in response to the subliminally presented negative affective stimuli, but five contrasts yielded no differences. With respect to the other EDA parameters, two contrasts yielded a greater *SCR magnitude* after the negative affective stimuli compared to the control stimuli, whereas two contrasts did not. Furthermore, one contrast yielded a higher *SCR rise time* after the negative affective stimuli compared to the control stimuli. Finally, another contrast showed that the mean *SCR frequency* was equal after both stimulus types.

Conclusion and recommendations. All in all, in 30 of the 48 (63%) contrasts EDA responses increased to subliminally presented negative affective stimuli relative to the control stimuli. These significant differences were mostly found in studies using SCR amplitude as the EDA parameter, fear conditioning as the experimental paradigm, and fear-relevant stimuli. The inconsistencies in the results can be attributed to several factors. In general, a great variation is apparent between studies in determination of the time window of interest, apparatus, and statistical data transformations. For example when determining SCR amplitude, individual differences should be taken into account by applying a range correction should be applied (184). This is a procedure in which the SCR of the individuals is expressed in the maximum and minimum level of response amplitude. However, not all studies performed this correction. Additionally, there are substantial differences in the quantification of the various EDA responses (103). For example GSR was expressed in number of responses but also in amount of (in)correct responses, and the time window in which SCR is expected to increase compared to a baseline was different across studies (see Table 5). Furthermore, room temperature and humidity have been found to affect EDA. For example SCR amplitude can increase with a 1° increase in room temperature (for an overview see 185). However, none of the studies reported on these factors. Finally, not all studies reported on the handling of nonresponders. Since part of the general population might not show EDA responses (e.g., 186), it should be explicitly addressed whether the data of these nonresponders were omitted or not. This was generally not the case in the included studies.

In conclusion, the reviewed literature suggests that negative affective stimuli that are presented below threshold of awareness might increase EDA responses compared with control stimuli, but this effect might be limited to stimuli that are 'biologically fear-relevant' (187), such as snakes and spiders.

TABLE 5 Study outcomes for electrodermal responses (i.e., galvanic skin response (GSR), Skin conductance level (SCL), Skin conductance response (SCR)) per manipulation type

Fear conditioning studies					
Author	*Year*	*Units of analysis*	*Results*	*Effect size*[1]	*Quality (1-15)*
Aderman et al.	1964	GSR: Number of correct and incorrect responses based on substantial deflection[2]	CS+ > CS-	-	9
Beisgen and Gibby	1969	GSR: Correct and incorrect responses	CS+ ≠ CS-	-	9
Cornwell et al.	2007	SCR: Magnitude, max. deflection initiated at 500-5000 ms after onset of stimulus > 0.03 µS, averaged per CS	CS+ = CS-	-	13
Corteen and Wood	1972	SCL: Number of responses based on change > 1 K ohm, within 3 s after CS	CS+ > CS-	-	9
Flo et al.	2011	SCL: Change > 0.03 µS within 1 - 5 s after CS, with a subsequent reduction in mV of 1/3 peak value	Across groups[3]: S2 (extinction): CS+ > CS- REM (extinction): CS+ > CS-	 η_p^2 = 0.28 η_p^2 = 0.22	7
Flykt et al. (study 1)	2007	SCR: Amplitude, max. deflection initiated within 1 - 4 s after CS onset > 0.032 µS. Applied a range correction[4]	Snakes: CS+ > CS- Guns: CS+ > CS-	*d* = 0.30 *d* = 0.18	12
Flykt et al. (study 2)	2007	SCR: Amplitude, max. deflection initiated within 1 - 4 s after CS onset, > 0.032 µS. Applied a range correction	CS+ = CS-	-	12
Lazarus and Mc Cleary	1951	SCR: Amplitude, averages of GSR's over 5 s after CS	CS+ > CS-	*d* = 2.48	7
Lipp et al.	2014	SCR: Magnitude, max. response initiated within 4 - 7 s after CS onset, averaged across the four trials per CS+ and CS- in each block	CS+ > CS-[5]	η_p^2 = 0.43	11
Núñez and De Vicente (study 1)	2004	SCR: Amplitude, within 4 s after the CS+. Applied a range correction	n.r.	-	8
Núñez and De Vicente (study 2)	2004	SCR: Amplitude, within 4 s after the CS+. Applied a range correction	n.r.	-	8
Öhman and Soares (study 2)	1993	SCR: Amplitude, max. deflection initiated within 1 - 4 s after CS, < 0.05 µS. Square root transformed. Applied a range correction	Fear-relevant: CS+ > CS- Fear-irrelevant: CS+ = CS-	*d* = 0.66 -	11

2

Author	*Year*	*Units of analysis*	*Results*	*Effect size*[1]	*Quality (1-15)*
Öhman and Soares (study 3)	1993	SCR: Amplitude, max. deflection initiated within 1 - 4 s after CS, < 0.05 µS. Square root transformed. Applied a range correction	Fear-relevant: CS+ > CS- Fear-irrelevant: CS+ = CS-	*d* = 0.94 -	11
Öhman and Soares (study 1)	1998	SCR: Amplitude, max. deflection initiated within 1 - 4 s after CS, < 0.05 µS. Square root transformed. Applied a range correction	Acquisition: CS+ > CS-, in fear-relevant group only	*d* = 1.44	10
Öhman and Soares (study 2)	1998	SCR: Magnitude, max. deflection initiated within 1 - 4 s after CS, < 0.05 µS. Averaged per block of four trials. Square root transformed. Applied a range correction	In all groups[6]: Acquisition: CS+ > CS- Extinction: n.r.	η_p^2 = 0.58	12
Olsson and Phelps	2004	SCR: Peak-to-peak amplitude difference to the first response within 0.5 - 4.5 s after CS onset, > 0.02 µS. Square root transformed	In all groups: Acquisition: CS+ > CS-, in Pavlovian group only Extinction[7]: CS+ = CS-	- -	12
Parra et al. (study 1)	1997	SCR: Amplitude of the largest response measured initiated within 1 - 4 s after CS onset on the unreinforced trials. Applied a range correction	CS+ > CS-	*d* = 0.40	10
Parra et al. (study 2)	1997	SCR: Amplitude of the largest response measured initiated within 1 - 4 s after CS onset on the unreinforced trials. Applied a range correction	Acquisition: CS+ > CS- Extinction: CS+ > CS-	η_p^2 = 0.38 *d* = 0.40	11
Peper and Karcher	2001	SCR: Amplitude, max. deflection within 1 - 4 s after stimulus onset, > 0.05 µS, Log (+1) transformed. Applied a range correction	n.r.	-	10
Saban and Hugdahl	1999	SCR: Amplitude, responses within 1 - 4 s after CS onset, > 0.004 µS during 100 ms epochs. Applied a range correction.	n.r.	-	10
Soares and Öhman	1993	SCR: Amplitude, max. deflection initiated within 1 – 4 s after a CS, > 0.05 µS. Square root transformed. Applied a range correction	Fear-relevant CS: CS+ > CS- Neutral CS: CS+ = CS-	*d* = 0.71 *d* = 0.61[8] -	9

Author	Year	Units of analysis	Results	Effect size[1]	Quality (1-15)
Tassinary et al.	1984	SCR: Amplitude, within 1.5 s prior to CS onset, ending 4 s later. Phasic response scores were converted into z-scores	CS+ < CS-	η_p^2 = 0.47	7
Wall and Guthrie	1959	GSR: 10 stimulus presentations coded + or - relative to their median GSR	CS+ > CS-	-	5
Wardlaw and Kroll	1976	SCL: A response occurred with a change of at least 1 K ohm, within 3 s after CS	CS+ = CS-	-	10
Wiens et al.	2003	SCR: Magnitude of first response initiated within 0.9 - 4 s after CS onset. Square root transformed	CS+ > CS[9]	η_p^2 = 0.26	10
Wong et al.	1994	SCR: Change in SCL (delta C) > 0.1 μmho between prestimulus level (400 ms) and within 1 - 4 s after CS. Square root transformed	Frequency: CS+ > CS- Amplitude: CS+ > CS- Latency: CS+ = CS- Magnitude: CS+ = CS-	*d* = 0.45 *d* = 0.39 - -	9
Worthington (study 1)	1966	GSR: Mean % changes (changes within 1 - 4 s after CS onset) of first and last 6 unreinforced CS presentations	n.r.	-	10
Worthington (study 2)	1966	GSR: Mean % changes (changes within 1 - 4 s after CS onset) of first and last 6 unreinforced CS presentations	Acquisition: CS+ > CS-	*d* = 1.13	10
Priming studies					
Author	*Year*	*Units of analysis*	*Results*	*Effect size[1]*	*Quality (1-15)*
Codispoti et al. (study 1)	2009	SCR: Amplitude, max. change within 1 - 4 s after stimulus onset. Log transformed	Unpleasant = pleasant[10]	η^2 = 0.13	7
Codispoti et al. (study 2)	2009	SCR: Amplitude, max. change within 1 - 4 s after stimulus onset. Log transformed	< 80 ms[11]: Unpleasant = neutral and pleasant	-	7
Kimura et al.	2004	SCR: Amplitude, max. deflection within 1 - 4s after stimulus offset, > 0.05 μS. Log transformed (+ 1)	n.r.	-	11
Lapate et al.	2014	SCL: Amplitude, change within 1- 4 s after stimulus onset, > 0.02 μS. Square root transformed	Fearful faces > neutral Spiders = neutral	*d* = 0.32 *d* = 0.19	13

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Author	Year	Units of analysis	Results	Effect size	Quality (1-15)
Lee and Tyrer	1981	SCL: Amplitude, n.r.	n.r.	-	11
Najström and Jansson	2007	SCL: Amplitude, distance between lowest and highest response within 1 s before and 11 after stimulus presentation. Square root transformed	Threat > neutral	*d* = 0.22	12
Nielsen and Kaszniak[12]	2006	SCR: Magnitude, first SCR within 1 - 4 s after stimulus onset, > 0.03 µS	Threat = neutral Threat = pleasant	-	11
Reagh and Knight	2013	SCR: Amplitude, max. response during 10 s after startle presentation compared to baseline (response onset), > 0.05 µS	Negative > neutral Negative > happy	*d* = 1.15 *d* = 1.16	12
Silvert et al.	2004	SCL: Magnitude, changes within 1 - 4 s after stimulus onset, averaged for each type of word. Square root transformed	Negative > neutral	*d* = 0.65	11
Tan et al. (study 3)	2013	SCR: Magnitude, max. change during 1 – 5 s after stimulus onset. Log transformed (+ 1)	Negative > neutral	$\eta^2 = 0.16$	13
Williams et al.	2006	SCR: Amplitude, change within 1 - 3 s after stimulus offset compared with baseline, > 0.05 µS	Fear = neutral	*d* = 0.49	12
Williams et al.	2004	SCR: Amplitude, change within 1 – 3 s after target offset compared with baseline, > 0.05 µS	Amplitude: Fear = neutral Frequency: Fear = neutral Rise time: Fear > neutral[13]	- - *d* = 0.49, 0.66	9

Note. *Abbreviations*: GSR = Galvanic skin response, CS = Conditioned stimulus, US = Unconditioned stimulus, CS+ = CS paired with US, CS- = CS not paired with US, SCR = Skin conductance response, ms = Milliseconds, µS = MicroSiemens, SCL = Skin conductance level, K ohm = Kilo ohm (resistance), s = Seconds, mV = Millivolt, S2 = Stage 2 (sleep stage), REM = Rapid eye movement (sleep stage), n.r. = Not reported, µrho = Microrho (density).

1 Effect sizes are displayed in η^2,η_p^2 or *d* as appropriate, but are not always available due to missing information (e.g., cell sizes).

2 Deflection is a common used term for 'change in SCL' (185).

3 Group 1: US: Images with negative emotional valence, CS: Neutral sound; Group 2: US: Mild electric shock, CS: Neutral sound; Group 3: Aversive shocks during sleep.

4 Range correction: For each individual the SCRs were divided by the largest response amplitude recorded during the experiment to reduce irrelevant variation caused by differences in reactivity between subjects (184).

5 Independent of the nature (snakes or wallabies) of the CS+.

Continues >

Electromyographical (EMG) activity. Surface EMG measures the electromagnetic field at the surface of the skin (for a description of the outcome measures the reader is referred to 188). The findings are displayed in Table 6 and operationalizations of the EMG measure are reported for each study.

Fear conditioning studies. In three fear conditioning studies EMG was measured and three contrasts were tested. One contrast yielded a higher response of the *corrugator supercilii* to the CS+ compared to the CS-. Another contrast did not find this difference, but in that specific study the exposure time to the target was exceptionally low (2 ms; 131). Furthermore, one contrast showed a higher *orbicularis oculi* response to the CS+ compared to the CS-.

Priming studies. In 13 priming studies, testing 40 contrasts, EMG was measured. Where eight contrasts found higher activity of the *corrugator supercilii* in response to negative affective stimuli compared to the control stimuli, ten other contrasts did not find this difference. A similar pattern was found for *zygomatic major* activity as for the corrugator supercilii but reversed (as expected): five contrasts indicated lower zygomatic major activity in response to the negative affective stimuli compared to the control stimuli, whereas nine contrasts did not find a difference. Furthermore, three contrasts testing *orbicularis oculi muscles* activity showed a higher peak response to negative affective stimuli compared to the control stimuli, but three other contrasts did not indicate any differences between stimulus types. Finally, two contrasts indicated that *general EMG activity* was not different between the stimulus types. Importantly, Codispoti, Mazzetti, and Bradley (2009, 133) found a significant difference in *corrugator supercilii* activity at a stimulus presentation of 50 ms, but not at faster presentation times (i.e., 25 ms and 40 ms). Additionally, Sonnby-Borgström, Jönsson, and Svensson (2008, 168) found an effect of presentation duration on the *zygomatic major* activity; negative affective stimuli elicited a smaller response than the control stimuli, but only when presented for 17-23 ms and not for 65-70 ms.

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[6] Groups differed in received instructions on the goal of the study, but the overall pattern was the same.

[7] Groups comprised of either Pavlovian learning, observational learning, or instructed learning.

[8] At the start of extinction half of the subjects were informed that no more shocks would be administered and other half was not informed. Effect size is reported for these respective groups.

[9] Omnibus analysis of variance that includes the supraliminal condition. A significant interaction was found for condition, $F(3,81) = 9.50$, but the main effect of condition was not significant, $F < 1$.

[10] Exposure durations: 25 ms, 80 ms, 250 ms, 500 ms, 1500 ms, 2000 ms, 5000 ms, and 6000 ms.

[11] Exposure durations: 25 ms, 40 ms, 50 ms, 80 ms, 150 ms, 250 ms, and 1000 ms.

[12] Results are reported for the control group only.

[13] Exposure durations: 30 ms and 10 ms are reported, respectively

Conclusion and recommendations. Together, 18 of the 41 contrasts (44%) reported hypothesis congruent results for the measured EMG response (i.e., increases to subliminally presented negative affective stimuli compared with control stimuli). Although the results for the different types of EMG measures are mixed, the findings point toward an increase of EMG activity after subliminal negative affective stimuli (or a decrease in the case of smiling). There are some possible explanations for the mixed results. One main issue in EMG measurement is the relatively subtle signals it produces and its high susceptibility to noise from task-unrelated sources. Sufficient noise reduction, attention to electrode sizes, and appropriate signal conditioning techniques should be employed (188). In the reviewed literature, most of the studies corrected for noise and reported details on measurements and data reduction. However, some studies merely reported that “the measurement had been performed and analyzed” (165,170). Furthermore, zero signal baselines are hard to obtain for EMG measures since muscle activity is rarely absent. More importantly, placing electrodes on facial muscles might already influence participants to show unnatural responses (188). In sum, from the current literature it cannot be concluded that negative affective stimuli below threshold of awareness differentially influence EMG compared with control stimuli.

TABLE 6 Study outcomes for electromyographical measures per manipulation type

Author	Year	Units of analysis	Results	Effect size[1]	Quality (1-15)
Fear conditioning studies					
Bunce et al.	1999	Corrugator supercilii: Mean change from baseline (400 ms before CS onset) in response to stimulus. Log transformed	CS+ = CS-	*d* = 0.04	12
Golkar and Öhman	2012	Startle: Mean startle magnitude to startle probe between trials	CS+ > CS-	$\eta^2 = 0.44$	12
Tassinary et al.	1984	Corrugator supercilii: Averaged over 0.5 s intervals from 1.5 s prior to CS and 4 s after,	CS+ > CS-	$\eta_p^2 = 0.47$	7
Priming studies					
Author	*Year*	*Units of analysis*	*Results*	*Effect size*[1]	*Quality (1-15)*
Bornemann et al.	2012	EMG activity: Mean change from baseline (200 ms before prime onset) over a 2 s interval	Zygomatic major: Anger = happy Anger = neutral Corrugator supercilii: Anger > happy Anger > neutral	 *d* = 0.13 *d* = 0.06 *d* = 0.55 *d* = 0.37	8
Codispoti et al. (study 1)	2009	Corrugator supercilii: Mean change from baseline (1 s before prime onset) over a 6 s interval	Unpleasant > neutral & pleasant, at all exposure durations[2]	$\eta^2 = 0.39$	7
Codispoti et al. (study 2)	2009	Corrugator supercilii: Mean change from baseline (1 s before prime onset) over a 6 s interval	25 & 40 ms[3]: Unpleasant = neutral & pleasant 50 ms: Unpleasant > pleasant Unpleasant > neutral	- $\eta^2 = 0.08$ $\eta^2 = 0.08$	7
Dimberg et al.	2000	EMG activity: Mean change from baseline (1 s before prime onset) averaged over 100 ms epochs during the first s of exposure	Zygomatic major: Anger < happy Anger < neutral Corrugator supercilii: Anger > happy Anger > neutral	 *d* = 0.68 *d* = 0.36 *d* = 0.56 *d* = 0.37	10
Lee and Tyrer	1981	Frontalis: N.r.	n.r.	-	11
Nielsen and Kaszniak[4]	2006	EMG activity: Mean change from baseline (1 s before prime onset) of 100 ms epochs during stimulus presentation (3 s)	Zygomaticus major: Unpleasant = neutral Unpleasant = pleasant Corrugator supercilii: Unpleasant = neutral Unpleasant = pleasant	-	11

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Author	*Year*	*Units of analysis*	*Results*	*Effect size*[1]	*Quality (1-15)*
Ravaja et al.	2004	EMG activity: Mean values of each of the first three 15-s epochs during the stimulus presentation	Zygomaticus major: Anger = happy Anger = neutral Corrugator supercilii: Anger = happy Anger = neutral Startle: Anger = happy Anger = neutral	 *d* = 0.13 *d* = 0.07 *d* = 0.08 *d* = 0.08 *d* = 0.13 *d* = 0.08	14
Reagh and Knight	2013	Startle: Mean peak response from baseline (50 ms before to 20 ms after startle probe onset) between 20 and 150 ms after startle probe onset	Negative > neutral Negative > happy	*d* = 0.95 *d* = 1.00	12
Rotteveel et al. (study 2)	2001	EMG activity: Mean over first 500 ms prior to trial onset and 3 s following prime onset	Zygomaticus major: Negative < positive Corrugator supercilii: Negative > positive	 *d* = 0.32 *d* = 0.28	12
Ruiz-Padial et al.	2011	EMG startle (orbicularis oculi muscle regions): Difference in mV between peak and onset of the response	Unpleasant > neutral Unpleasant = pleasant	*d* = 0.32 *d* = 0.13[5]	11
Smith	1993	EMG activity: Mean of the last three readings during presentation	Negative = positive Negative = neutral	*d* = 0.04 *d* = 0.09	10
Sonnby-Borgström et al.	2003	EMG activity: Mean strength from stimulus onset to 2500 ms after onset	Zygomaticus major: Anger = happy Corrugator supercilii: Angry = happy	 *d* = 0.18 *d* = 0.09	9
Sonnby-Borgström et al.	2003	EMG activity: Mean strength from stimulus onset to 2500 ms after onset	Zygomaticus major: Anger = happy Corrugator supercilii: Angry = happy	 *d* = 0.18 *d* = 0.09	9

Note. *Abbreviations*: CS = Conditioned stimulus, US = Unconditioned stimulus, CS+ = CS paired with US, CS- = CS not paired with US, s = Seconds, ms = Milliseconds, EMG = Electromyography, mV = Millivolts, n.r. = Not reported.

[1] Effect sizes are displayed in η^2,η_p^2 or *d* as appropriate, not always available due to missing information (e.g., cell sizes).

[2] Exposure durations: 25 ms, 80 ms, 250 ms, 500 ms, 1500 ms, 2000 ms, 5000 ms, and 6000 ms.

[3] Exposure durations: 25 ms, 40 ms, 50 ms, 80 ms, 150 ms, 250 ms, and 1000 ms.

[4] Results are reported for the control group only.

[5] Omnibus analysis of variance that includes the supraliminal condition. A significant interaction was found for exposure duration and stimulus valence, $F(2,54) = 7.69$, but the main effect of exposure duration was not significant.

Continues >

Cardiovascular (CV) activity. CV activity can be measured in different ways depending on the parameter of interest (for an overview see 95). Results are displayed in Table 7 and the operationalizations of CV activity are reported for each study. Notably, one fear conditioning study measured changes in HR acceleration and deceleration, but did not report on the outcomes (156). No other fear conditioning studies measured CV activity.

Priming studies. In 15 priming studies CV activity was measured and 58 contrasts were tested. Seven contrasts indicated that the negative affective stimuli resulted in a higher *systolic blood pressure* (SBP) compared to the control stimuli while five contrasts yielded no differences between the stimulus types. A higher *diastolic blood pressure (DBP)* to negative affective stimuli than to control stimuli was found in one of the ten contrasts. *Heart rate* (HR) for negative affective stimuli compared with control stimuli was higher in one contrast, but in 17 contrasts the HR was equal for both stimulus types. Five contrasts indicated a lower *pre-ejection period* (PEP) in response to negative affective stimuli compared to control stimuli, but three contrasts yielded no differences. The other CV parameters, *heart rate variability* (HRV), *total peripheral resistance* (TPR), *ventricular contractility* (VC), *cardiac output* (CO), *respiratory sinus arrhythmia* (RSA), and *respiratory rate* (RR) were only tested in a handful of studies. Two contrasts yielded no differences between stimulus types in HRV. One contrast indicated a higher, instead of the expected lower, RSA after negative affective stimuli and two other contrasts did not find a difference in RSA between stimulus types. One contrast found a higher TPR was found in response to negative affective stimuli compared to control stimuli and one other contrast found a lower CO. Finally, there were no differences between stimulus types on VC or RR.

Importantly, in two cases an increase of SBP reactivity was reported for sad faces, but not for angry faces, compared to the control stimuli. In four contrasts that yielded SBP increases the word 'angry' was used as a prime. In one contrast sad faces were used as a prime, which yielded higher SBP. However, another contrast did not indicate a difference between sad and control faces. Similarly, PEP responses to negative affective stimuli compared to control stimuli were found in response to sad and fearful faces, but not in response to angry faces, although one other contrast indicated that sad facial stimuli resulted in a higher PEP.

Conclusion and recommendations. Overall, 14 out of 58 contrasts (24%) indicated increased CV activity in response to subliminal negative affective stimuli compared with the control stimuli. The results suggest that some of the CV effects are related to the specific valence of the stimuli, resulting for example in different outcomes for 'angry' stimuli and 'sad' stimuli. However, the findings differed strongly across the

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[6] Omnibus analysis of variance that includes the supraliminal condition. No significant interactions or main effects are reported.

various CV parameters. Still, the most consistent results emerged for SBP; it was larger in response to negative affective stimuli in seven out of 13 contrasts. In contrast, HR and DBP did not respond to specifically to negative affective stimuli. For PEP mixed results were apparent. Furthermore, for other parameters too few studies were found to summarize the findings. The ambiguity of the findings can be attributed to several methodological issues. Biobehavioral variables are often not reported on, such as recent eating, drinking, medication use and exercise prior to the study, while these factors influence the results greatly (189). Additionally, between-subjects comparison of impedance cardiography based outcome measures (e.g., CO, PEP, and TPR) is controversial; the absolute values are prone to various influences and the comparisons might not represent the state of the organism. To overcome this, within-subject designs are preferred (190). Here, this was only done by Garfinkel et al. (2016, 61); others studies used a between-subjects design. Notably, in this specific area of subliminal presentation CV measures are apparently rarely taken into account in fear conditioning studies.

In conclusion, with the exception of effects on SBP, little consistency is found in the literature concerning the effect of subliminally presented negative affective stimuli compared with control stimuli. These mixed findings are likely due to a wide variety of outcome measures based on different physiological features and measurement properties.

TABLE 7 Study outcomes for cardiovascular measures separately per manipulation type

Author	Year	Units of analysis	Results	Effect size[1]	Quality (1-15)
Fear conditioning studies					
Peper and Karcher	2001	Accelerative HR changes: Max. HR 3 - 10 s after stimulus onset relative to the mean HR within a 10 s prestimulus interval. Deceleration HR changes: 0.5 - 3 s after stimulus onset	n.r.	-	10
Priming studies					
Chatelain and Gendolla (study 1)	2015	PEP, HR, SBP, and DBP: : Averages of last 4 min baseline subtracted from values during the priming and averaged over 1 min periods	PEP: Fear > happy Anger = happy HR: Fear = happy Anger = happy SBP: Fear = happy Anger = happy DBP: Fear = happy Anger = happy	 *d* = 0.92 *d* = 0.33 *d* = 0.42 *d* = 0.06 *d* = 0.11 *d* = 0.32 *d* = 0.31 *d* = 0.20	12
Garfinkel et al. (study 1)	2016	SBP, HR, and HRV: Baseline (2000 ms preceeding stimuli block) subtracted from averages of blocks of trials	SBP: Anger > relax HR: Anger = relax HRV: Anger = relax	*d* = 0.57 *d* = 0.01 *d* = 0.17	12
Garfinkel et al. (study 2)	2016	SBP, HR, and HRV: Baseline (2000 ms preceeding stimuli block) subtracted from averages of blocks of trials	SBP: Anger > relax HR: Anger = relax HRV: Anger = relax	*d* = 0.61 *d* = 0.25 *d* = 0.41	13
Gendolla and Silvestrini (study 1)	2011	PEP, SBP, DBP, and HR: Averages over 1 min periods. CV reactivity (average baseline values over last 4 min of habituation minus average values during the task)	PEP: Anger = happy Sad < happy SBP: Angry = happy Sad > happy DBP: Angry = happy Sad = happy HR: Angry = happy Sad = happy	 *d* = 0.04 *d* = 0.04 - - - - - -	13

Author	*Year*	*Units of analysis*	*Results*	*Effect size*[1]	*Quality (1-15)*
Gendolla and Silvestrini (study 2)	2011	PEP, SBP, DBP, and HR: Averages over 1 min periods. CV reactivity (average baseline values over last 4 min of habituation minus average values during the task)	PEP: Anger = happy Sad < happy SBP: Angry = happy Sad > happy DBP: Angry = happy Sad = happy HR: Angry = happy Sad = happy	 *d* = 0.07 - - - - - - -	13
Hull et al. (study 3)	2002	SBP, DBP, and HR: Average of two readings at each measurement period[2]	SBP: Angry > relax DBP: Angry > relax HR: Angry = relax	$\eta_p^2 = 0.12$ $\eta_p^2 = 0.15$ $\eta_p^2 = 0.08$	8
Hull et al. (study 4)	2002	SBP, DBP, and HR: Average of two readings within each measurement period[2]	SBP: Angry > relax DBP: Angry = relax HR: Angry > relax[3]	- - $\eta_p^2 = 0.08$	8
Jönsson and Sonnby-Borgström	2003	RSA: During 5 min in each condition. HR: Averages over 2 s before (baseline) and 7.5 s after onset of the stimulus	RSA: 17 ms: Angry > happy 56 ms: Angry = happy HR: 17 ms: Angry = happy 56 ms: Angry = happy	 *d* = 0.18 *d* = 0.04 *d* = 0.01 *d* = 0.04	10
Kemp-Wheeler and Hill	1987	HR and RR: Average of last 15 s prior to stimulus onset (baseline) compared with 15 s after stimulus offset	HR: Negative = neutral RR: Negative = neutral	-	11
Lasauskaite et al.	2013	PEP: Averages of last 5 min baseline subtracted from task averages	Sad > happy	$\eta_p^2 = 0.17$	13
Lasauskaite Schüpbach et al.	2014	PEP, SBP, DBP, and HR: Averages of last 5 min baseline subtracted from task averages	PEP: Sad < happy[4] SBP: Sad = happy DBP: Sad = happy HR: Sad = happy	$\eta^2 = 0.03$ - - -	10
Lee and Tyrer	1981	HR, RR, and BP: N.r.	n.r.		11
Ravaja et al.	2004	RSA: Average of 45 s divided in 15-s epochs during primes. Natural logarithm was applied	Anger = happy Anger = neutral	*d* = 0.06 *d* = 0.04	14
Ruiz-Padial et al.	2011	HR: Task minus baseline in 0.5 s increments	Unpleasant = neutral Unpleasant = pleasant	- -	11

Author	Year	Units of analysis	Results	Effect size[1]	Quality (1-15)
Silvestrini and Gendolla	2011a	PEP, HR, SBP, DBP, and TPR: Averages of last 4 min of baseline subtracted from averages during the task	PEP: n.r. SBP: n.r. DBP: n.r. TPR: n.r.		11
Silvestrini and Gendolla	2011b	HR, SBP, and DBP: One min averages of last 2 min of baseline subtracted from those during the task	SBP: Sad > happy DBP: Sad = happy HR: Sad = happy	*d* = 0.26 - -	11
Weisbuch-Remington et al. (study 1)	2005	HR, VC, CO, and TPR: Average from last min of rest period subtracted from average value first min of the task	HR: Negative = positive VC: Negative = positive CO: Negative < positive TPR: Negative > positive	- - *d* = 0.51 *d* = 0.51	10
Weisbuch-Remington et al. (study 2)	2005	HR, VC, CO, and TPR: Average from last min of rest period subtracted from average value first min of the task	HR: n.r. VC: n.r. CO: n.r. TPR: n.r.	-	10

Note. *Abbreviations*: HR = Heart rate, s = Seconds, n.r. = Not reported, PEP = Pre-ejection period, SBP = Systolic blood pressure, DBP = Diastolic blood pressure, HRV = Heart rate variability, ms = Milliseconds, CV = Cardiovascular, RSA = Respiratory sinus arrhythmia (HF power), BP = Blood pressure, VC = Ventricular contractility, CO = Cardiac output, TPR = Total peripheral resistance.

[1] Effect sizes are displayed in η^2,η_p^2 or *d* as appropriate, not always available due to missing information (e.g., cell sizes).

[2] Baseline, after instructions, after practice trials, after the priming task, and during recovery.

[3] For HR *N* = 32.

[4] The study entailed a difficult and easy condition, but there were no differences between the conditions

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Quality of the studies

The quality of the studies ranged from 5 to 14 out of a possible 15 points (Table 5 through 7) with a median quality rating of 10. Clearly, year of publication was of influence; most of the studies with a rating of nine or lower were published before 2000. This is understandable since science is not a static entity; some of the items are only recently considered to be vital, such as statements on conflict of interest or ethical approval. However, there are some exceptions. For example Flo et al. (2011, 137) scored a seven. The authors did not report on a blinding procedure of the researchers, blinded allocation to conditions, or an awareness check, means and standard deviations or other estimates of variation in the sample, and omitted results for some of the outcome measures. Overall, studies in this field could improve greatly by reporting effect sizes or providing the statistics and final cell sizes to be able to calculate the effect sizes enabling the execution of meta-analyses (191). Additionally, the current results highlight the need of reporting sufficient detail about the study performed, the measures used, methods for data reduction, and agreement on these matters within the field. Together, these improvements would enable a more constructive scientific approach and accumulation of knowledge.

Discussion

With this systematic review we set-out to find all articles discussing empirical studies using subliminal negative affective stimuli while measuring the effect on CV, EDA, EMG, hormonal, and immunological activity in a healthy sample. We hypothesized that subliminally presented negative affective stimuli would increase peripheral physiological activity compared with a positive or neutral affective state. Overall, 60 (41%) of the 147 reported contrasts based on 65 studies revealed the expected effect, while four (2.7%) of the reported contrasts showed an opposite effect. The remaining portion (56%) did not find an effect. No studies were found that reported hormonal and immunological outcomes.

Within these mixed results some consistent findings were apparent. In fear conditioning studies, the expected effect was found relatively consistent for all EDA parameters while the other physiological outcomes have not been tested extensively. In the priming studies, the findings were mixed for the EDA parameters; there was a general absence of effects for SCR amplitude while some effects on SCR magnitude were apparent. Considering CV activity, a rather consistent effect on SBP was found but not on HR and DBP. No effects were found for EMG activity. However, little consistency in methodology was found across studies. There were major differences in stimulus types used (e.g., faces, words, with differences in valence), data handling, and outcome reporting. Furthermore, some outcome measures, such as PEP and TPR, have been understudied in this area. Not surprisingly, the included studies and outcomes have not previously been interpreted in terms of relevance to health. In sum, from the available literature it cannot be firmly concluded that subliminally presented negative affective stimuli affect peripheral physiological activity. The findings are inconsistent across the various physiological parameters and a low number of studies for each outcome measure has been performed. This warrants more and consistently conducted and reported research. These concerns will be addressed below.

The inconsistent results can be attributed to several factors. First, despite the use of comparable experimental methodologies (fear conditioning and priming, subliminal presentation of stimuli, and measurement of physiological parameters) the study designs and outcomes are framed within specific theoretical contexts. For example Gendolla and colleagues (e.g., 146,163,164) have focused on effort mobilization during a mental concentration task, which is expressed in PEP and is thought to be modulated by subliminally presented faces that convey an emotion. Similarly, Öhman and colleagues have focused on the underlying mechanisms of fear from an evolutionary perspective by using fear conditioning to change EDA (e.g., 187). Furthermore, Hull and colleagues (e.g., 62) focused on the dynamics of self-regulation and effects on CV activity in after priming. These different specific theoretical frameworks result in subtle methodological differences that limit comparability of the results. Furthermore, only a few (partial) replication studies were found (e.g., 172, replicating 135; 61, partially replicating 62). This suggests that only a handful of cases attempted to replicate previous findings before trying a variation in the study designs. Again, this is likely due to the different perspectives between studies; they simply were not meant to provide systematic scientific progress. To sum-up, the inconsistencies found are at least partly the consequence of our attempt to gather 'field-independent' evidence.

Furthermore, the abundance of differences in stimulus presentation is likely to have influenced the overall findings. One salient example is the use of fear-relevant versus fear-irrelevant stimuli in the fear conditioning studies. Öhman (2009, 187) reasoned that conditioned fear-relevant images (e.g., of snakes) lead to stronger physiological responses compared to conditioned fear-irrelevant images (e.g., of mushrooms) suggesting an evolutionary advantage of being aware of snakes and spiders rather than of flowers or mushrooms. However, Lipp et al. (2014, 148) reported a fear conditioning study using snakes and wallabies (which are inherently cute and nonfear inducing, according to the authors) as stimuli and the same effect was found for both stimulus types. Moreover, Lapate, Rokers, Li, and Davidson (2014, 144) found a larger physiological response in their priming study to fearful faces compared to neutral faces, but not to spiders compared to neutral images. These differences add to the inconsistency in the findings. However, several other factors regarding stimulus presentation should also be considered. The studies by Codispoti et al. (2009, 133), Jönsson and Sonnby-Borgström (2003, 167), and Sonnby-Borgström et al. (2008, 168) indicate that *different presentation durations* might be of influence (i.e., participants might process stimuli presented for 50 ms or longer to a larger extent than shorter presentation durations). Moreover, it has been suggested that sensitivity to different presentation durations differs to a certain degree between individuals (46). Furthermore, the results from Wiens, Katkin, and Öhman (2003, 174) suggest an effect of *trial order* in conditioning (i.e., participants might learn the order of presentation and not the CS-US association when presentation of the CSs is not random). Additionally, using *words versus images* in affective priming may influence the findings, which is thought to depend on the specific task performed during priming (e.g., 192). Also, images of faces displaying *different emotions* appear to elicit specific physiological responses. Moreover, the physiological responses to sad faces seems to differ from responses to angry faces. This was also found by the second study of Chatelain and Gendolla (2015, 132), which was not included in this review since it only used negative affective primes. Interestingly, a study by Verkuil et al. (2015, 193) measured sadness in a diary study and found that sadness interacted with gender in its effects on HRV over 24 hrs; while sadder women had a higher HRV, the opposite was true for men. This suggests that different emotional stimuli could elicit a different response between individuals (e.g., based on gender). A similar interaction was found by Jönsson and Sonnby-Börgström (2003, 141) in a study where gender was a predictor of the physiological response to angry faces. All in all, this suggests that used stimulus type and other design features can also affect the influence of negative affective stimuli presented outside of awareness on physiology.

Importantly, the type of awareness check used to test to what extend stimuli were successfully rendered subliminal also differed greatly. Since the advocacy by Merikle to more clearly define 'awareness' (1984, 121), it is considered appropriate to surpass subjective evaluations on awareness of the stimuli. Merely considering verbal report (e.g., asking 'could you see the image?' or 'do you hear something?') can be seen as an insufficient way to determine awareness of the stimuli. However, as Merikle and Daneman (2000, 194) indicated, it can be argued that objective measures can still be considered (partly) subjective as they are hindered by for example motivation and number of trials. Nowadays, the value of additional confidence ratings of awareness is recognized (122). Moreover, when considering the abundance of options and discussions on this matter, it is remarkable that several studies conducted after 1984 still only used verbal report. As a solution, Wiens and Öhman (2007, 51) advise to provide sufficient details about the checks performed (for a thorough discussion of this and related topics the reader is referred to 195-197). Overall, the methodological inconsistencies highlight the need for standardization of methods of subliminal priming and fear conditioning. Therefore, we strongly encourage the execution of systematic overviews of the literature in experimental psychology and debate on standardization of methodology.

To adequately interpret the findings of this systematic review some limitations should be kept in mind. First, we intentionally only included studies with a healthy sample to find the more general mechanisms that theoretically precede physical illnesses. Yet, research on the mechanisms of fear conditioning has also been performed with for example phobic individuals (for a review see 187). Since this group is thought be more vigilant to threatening information (198) the effects of the subliminal stimuli on physiological activity might be stronger compared to those of a healthy population. Thus, the restriction to healthy samples might have led to an underestimation of the effects.

Second, we included only studies that used stimuli that were negative affective for the general population, that is, not only negative affective for a specific subgroup of the general population. This excluded for example the studies performed by Levy, Hausdorff, Hencke, and Wei (2000, 199) who performed a subliminal priming study with positive and negative stereotypes of ageing in a sample of 60 years and older. Another excluded study was done by Carlisle et al. (2012, 200) in which the participants were primed with personally determined social ties that varied in positivity and negativity. In both studies an effect of the negative affective stimulus on CV activity was found. Thus, it could be that specific samples respond to specific stimuli or that personalized negative affective stimuli may lead to a stronger physiological response, which would underestimate the results presented here.

Third, we included studies that presented subliminal stimuli, but the effects of supraliminal stimuli (presenting stimuli in such a way that subjects are usually aware of them), or both, were not evaluated. It might have been more adequate to test the effects of supraliminal as well as subliminal stimuli together, since in reality they are highly likely to co-occur (66). This was done by some studies (e.g., 133), but we did not further review these results. Future research should consider taking both methods of presentation into account to test whether subliminally presented negative affective information adds to the explanation of physiological activity in addition to conscious processes.

Fourth, we did not find studies with cortisol as an outcome measure, which may be surprising. However, in studies using subliminal presentation it would be impossible to adequately measure cortisol and relate this to the very subtle and fast manipulations as the change in glucocorticoids is relatively slow (e.g., 201) compared with for example changes in blood pressure. Thus, we have included glucocorticoids as an outcome measure, but the absence of findings is not surprising when taking into account the characteristics of the measure and this specific set of studies.

Finally, aside from one study, we did not include unpublished findings. This is a common problem for any review, but it might be particularly unfavorable in this case since the effects of interest were often of secondary importance in the reviewed studies. Therefore, it is reasonable to expect that when analysis of secondary importance were not statistically significant the results are even less likely to be published. To summarize the limitations, on the one hand the findings may underestimate effects because they only apply to a general healthy population and to generally stressful stimuli, but on the other hand the findings may overestimate effects when the problem of unpublished results is larger than anticipated.

The current study has two specific strengths. We introduce an elaborate procedure to find all relevant articles by producing an extensive keyword-profile to transcend field-dependent terminology. Usually, a rather typical keyword profile such as described as the initial profile in the Methods would be considered sufficient to find articles for a (systematic) review or meta-analysis. However, using an elaborated and systematically expanded profile, which includes evaluating the additional relevance of possible keywords from an exhaustive list, results in a far more comprehensive overview of the literature. In this case, out of the 54 articles 51 were found using the final keyword-profile. In contrast, the initial keyword-profile had resulted in finding only nine of those articles. Furthermore, the three articles that we did not find with the keyword-profile either had no abstract or keywords and a nonspecific title or used author-specific terminology, and were found by checking the references of other included articles. Hence, without the elaborated keyword-profile we would have only reviewed 17% of the available literature on this topic. Evidently, regular keyword-profiles are insufficient and do not allow for a growing body of knowledge but rather a reinvention of the wheel within the limits provided by a theoretical paradigm. In sum, we strongly recommend using one final exactly formulated keyword profile that can be replicated by other researchers and used for later state-of-the-art-updates concerning the research question. Another strength of the current systematic review is the attempt to capture the quality of the studies. No standardized method to indicate the quality of experimental studies within the field of psychology has been previously incorporated in reviews. From the quality assessment it became apparent that studies often do not report essential information of the construct studied that is needed to estimate the effect or impact, replicate the findings, or perform meta-analyses. Thus, we advocate the implementation of a standardized quality assessment of psychological experimental research to stimulate adequate reporting of findings. Implementing a more comprehensive literature searching method combined with the exact key word formulation and standardized quality assessment would greatly enhance the impact and reliability of conclusions in the field.

To conclude, only part of the retrieved studies found an effect of subliminally presented negative affective stimuli on physiological parameters with the most convincing evidence emerging for SBP and SCR amplitude. However, the methodological differences and the insufficient number of studies for most parameters hinder firm statements about the effect. As research has demonstrated convincingly and consistently that information presented outside of awareness can affect brain activity and behavior, it seems pivotal to more systematically examine the possible contribution of processes outside of awareness on peripheral physiology to elucidate the relation between negative affect and health.